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NEUROBEHAVIORAL EFFECTS OF CARBON MONOXIDE (CO) EXPOSURE IN HUMANS: ELEVATED CARBOXYHEMOGLOBIN (COHb) AND CEREBROVASCULAR RESPONSES

FINAL REPORT

Vernon A. Benignus[1], Matthew L. Petrovick[2]

and James D. Prah[1]

May 19, 1989

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A two-channel cranial impedance plethysmograph (CIP) was designed and constructed as a noninvasive measure of brain blood flow (BBF) in man. The instrument was designed to reduce some of the problems with instability and difficulty of use found in earlier commercially-available models. The CIP has been previously validated against other measures of BBF.

During carboxyhemoglobin (COHb) formation, BBF is known to increase. When BBF increases it compensates for the reduced ability of the blood to carry oxygen in the presence of COHb. Fifteen men breathed carbon monoxide (CO) to produce increases in COHb values ranging from endogenous 18.4%. Increased COHb was significantly related to a relative increase in BBF ($p < 0.019$). Data from a similar experiment on dogs was obtained from Richard

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ABSTRACT Ctd.
Traystman for reanalysis and comparison to human results. Human and dog data did not differ significantly in the relation to increased COHb. Various tests demonstrated that the CIP is a reliable device. It was also shown that the information in the two CIP channels (left and right sides of the head) is redundant with respect to increased COHb. There was a substantial amount of scatter about the line of best fit. Some subjects (both humans and dogs) did not show increased BBF with high-level COHb. It was hypothesized that subjects who sufficiently increased BBF would not be behaviorally affected by COHb. Subjects whose BBF did not increase after exposure would not, hypothetically, have compensated and would therefore show behavioral impairment. The importance of testing the hypothesis with future work was emphasized.

FOREWORD

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EXECUTIVE SUMMARY

The present document contains (a) a brief review of the literature regarding increased brain blood flow (BBF) in response to elevated carboxyhemoglobin (COHb), (b) a description of an instrument to measure BBF in humans in a noninvasive manner and (c) a report of an experiment in which BBF was measured in a group of men with various COHb levels.

It is a well-established fact that, in dogs and sheep, BBF increases as COHb is formed. This statement originates from experiments involving invasive, direct measurement of blood flow in the arterial blood supply to the brain. The increased BBF is hypothesized to provide a compensatory mechanism for the reduction in oxygen-carrying capacity of blood when COHb is present. The BBF compensation is (in group averages) adequate to compensate for both the lost oxygen-carrying capacity and a shifted oxyhemoglobin dissociation curve.

If compensation were adequate in all subjects at all times and for all parts of the brain, then there should be no behavioral effects of COHb unless there is some other mechanism than hypoxia by which COHb produces behavioral effects. Review of behavioral experiments implies that below 20 - 30% COHb little reliable effect on average performance is noted in healthy, young men at rest, who are not simultaneously exposed to other toxicants. It has also been shown that oxygen consumption in the brain does not begin to decline until after about 20 - 30% COHb. Thus, there is apparent agreement between the behavioral literature and the compensatory BBF data.

An instrument was designed and constructed to measure BBF in man. The device is a cranial impedance plethysmograph (CIP). This method of measurement of BBF had previously been standardized against other, more traditional methods of BBF quantification by other researchers. The instrument and data reduction methods were tested in the present experiment. While no measure of BBF in ml/min can be made with the CIP, it is possible to calculate the relative change in BBF, R(BBF). This is a dimensionless unit which is commonly used in the BBF literature.

To test the method of BBF measurement, fifteen men breathed air and carbon monoxide (CO) mixtures from a Douglas bag. CO concentrations in parts per million (ppm) in the bag were calculated and mixed to produce COHb levels ranging from endogenous to 19%. The BBF was measured before and after exposure and R(BBF) was computed for each subject. No behavior was measured.

The R(BBF) increased as a function of COHb. The slope and intercept of the regression function in humans were compared to a function fitted to data from Richard Traystman in dogs. There was no significant difference between the two functions. A single function was fitted to the combined dog and human data.

A notable feature of the R(BBF) data was its wide scatter about the line of best fit. Some subjects did not exhibit compensatory increases while others appear to have overcompensated. This was true of both humans and dogs. It was hypothesized that those subjects not compensating for COHb might

be behaviorally impaired by the COHb. The subjects who do compensate would show reduced or no behavioral effects. If this hypothesis were demonstrated, it could lead to the description of a population of subjects who are especially sensitive to behavioral impairment by COHb. No test of this hypothesis could be performed with the present data set.

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BBF	Brain blood flow. Also see R(BBF).
CIP	Cranial impedance plethysmograph, an instrument or its output which measures an analog of brain blood flow by measuring the cranial electrical bioimpedance changes.
CO	Carbon monoxide, an invisible, odorless gas which is the product of incomplete combustion.
COHb	Carboxyhemoglobin, a measure of the level of carbon monoxide in the blood.
p	Probability of an event or outcome of a test of statistical significance.
R(BBF)	Relative change in brain blood flow.
ppm	Parts per million.
r	Correlation coefficient (Pearson product-moment).

INTRODUCTION

The present document contains (a) a discussion of the data regarding increased brain blood flow (BBF) in response to elevated carboxyhemoglobin (COHb), (b) a description of an instrument to measure an analogue of BBF in humans in a noninvasive manner and (c) a report of an experiment in which an analogue of BBF was measured in a group of men with various levels of COHb.

BBF RESPONSE TO ELEVATED COHb

The Compensatory Mechanism Hypothesis. The most definitive and quantitative information about the BBF response to COHb is contained in a review and summary of a series of experiments performed in Richard Traystman's laboratory (Jones & Traystman, 1984). From this work, involving invasive, direct measurement of flow in the brain arterial supply in dogs and sheep, it appears that as COHb increases there is a corresponding proportional increase in BBF. The rise in BBF is apparently due to brain vascular dilation.

As COHb increases, the blood's ability to carry oxygen (O_2) is diminished in direct proportion. The effect of the increased BBF is to tend to compensate for the reduced O_2-carrying capacity of the blood. Thus, the BBF response to increased COHb can be teleologically regarded as a compensatory mechanism.

When COHb increases, not only is the O_2-carrying capacity of the blood reduced, but the O_2 dissociation curve is also shifted to the left (Lambertsen, 1980). The effect of the shift in the dissociation characteristic is to decrease the amount of O_2 which

is unloaded from the blood into the tissue (Lambertsen, 1980). If a compensatory mechanism is to be adequate to maintain an unchanged O_2 delivery to the tissues, then BBF must rise by more than the decreased O_2-carrying capacity due to COHb because there must be sufficient additional O_2 delivered to also compensate for the more difficult O_2 unloading.

The issue of adequacy of the compensatory BBF mechanism has also been addressed by Jones and Traystman (1984). Two lines of argument are offered as evidence that the compensation is sufficient to maintain unchanged O_2 supply to tissue even in the face of 20 - 30% COHb. First, the mean increase in BBF which was observed in subjects was larger than needed to compensate for the reduced O_2-carrying capacity, thus possibly reflecting a change great enough to also compensate for the increased difficulty in unloading O_2 from blood to tissue. Second, the mean O_2 consumption of the whole brain was not observed to fall for elevated COHb values in the above range. Jones and Traystman therefore hypothesized that the brain vasodilation is the effector part of a closed-loop O_2 regulation system.

Implications of Compensatory Mechanism. It is potentially important to study BBF compensation for COHb elevation in humans. If whole-brain O_2 delivery is regulated at the tissue level in the face of increasing COHb, brain function should not be impaired. The preceding statement rests upon a number of assumptions which are explicated and discussed below, but which have not been tested.

Assumption 1: Regulation of O_2 supply at the tissue level is homogenous across all regions of the brain. This is not likely to be an exactly accurate assumption. Even if the assumption were not valid in detail, but deviations in regulation were small, the function of brain regions might not be disturbed because of redundancies within the regions.

Differences in vasodilatory responses to COHb across brain regions were reported in sheep (Koehler et al., 1984) and in cats (Okeda et al., 1987). It is possible that the differences in regional BBF response to COHb were proportional to O_2 utilization rate and therefore appropriate to the demand. However, the BBF in one area of the brain, the neurohypophysis, has been shown to be unresponsive to COHb (Hanley et al., 1986; Wilson et al., 1987). Too little data are available to assess the implications of the findings or the effect of deviations from the assumption. To the extent that the above assumption is violated to an important degree, behavioral effects of carbon monoxide (CO) could occur despite compensatory action.

Assumption 2: Regulation remains effective over the entire exposure duration. A number of other compensatory mechanisms do not behave in a the above manner, e.g. the brain vasoresponse to hypoxic hypoxia (Krasney et al., 1984; Manohar et al., 1984) and for reduced carbon dioxide (CO_2), (Albrecht et al., 1987; Raichele et al., 1970). The BBF response in the above cases declined over the course of hours or days, depending upon the conditions. It is not, however, clear that the decline of the BBF response represents a failure of compensatory mechanisms so

much as it represents some other, more long-term adaptive mechanism coming into play. No data on the duration of the BBF response to COHb elevation are available.

Assumption 3: Regulation is equally effective for all subjects and all occasions of measurement. Again, there are no relevant data for CO in the peer-reviewed literature.

Assumption 4: There would be a behavioral decrement if there were a decrease in O_2 supply. It is not certain that a decrement in brain function would occur if the O_2 supply were slightly decremented. Brain tissue could increase its O_2 extraction to further compensate. No data are available on this possibility.

Assumption 5: The neurotoxic effect of CO exposure is entirely due to the hypoxic consequences of COHb formation. There is evidence that CO hypoxia is not the only mechanism by which CO produces effects (Piantadosi et al., 1987). The latter is apparently important only at high CO concentrations, however. If other toxic mechanisms played an important role, the BBF compensatory mechanism might be only partly effective. Current evidence seems to indicate, however, that other mechanisms have only very small effects under conditions of exposure of interest in this document.

BBF COMPENSATION AND BEHAVIORAL EFFECTS.

Benignus et al. (1989) reanalyzed reports of behavioral effects of COHb elevation. They concluded that the effects of COHb less than ca. 20% on brain function were small or absent. The conclusion was limited to normal, healthy, young males, who were not simultaneously exposed to other toxicants, under minimal

physical work conditions. This is the conclusion which would be expected from the compensatory mechanism hypothesis and data as outlined above (if all assumptions are valid) because brain O_2 supply is held constant by the compensatory mechanism and the O_2 consumption does not fall below 20 - 30% COHb.

The conclusion of Benignus et al. (1989) was tempered by some less formal observations. It appears that in nearly all of the reports of behavioral effects of elevated COHb, there were slight (but not statistically significant) elevations of mean behavioral error scores for COHb values less than 20%. It also appears that if data from individual subjects are examined, some subjects demonstrated decrements in performance while others did not or did so to a lesser extent (authors' own observations and those of Steven Horvath, 1989). The latter observation could explain the slight elevations in mean behavioral error scores and produce a high variance among subjects. If the above less formal observations were true, the conclusions of Benignus et al. (1989) should be re-stated. It is possible that some subjects respond to COHb elevation while others do not, or respond some of the time and not other times. Thus, it is possible that in the individual, if not in the group means, the effects of COHb elevation could be large and important.

The compensatory vasodilation hypothesis (above) is based upon data from group averages, as are behavioral conclusions. Assumptions made about the compensatory process regarding equal effects for all subjects and constant effects over time (assumptions number 2 and 3) may not hold. If either or both of

the assumptions do not hold, some subjects may not fully compensate at all times and thereby show behavioral effects of COHb elevation. No data are available to assess the validity of the various assumptions and it was therefore considered important to devise and test a method to collect such data in a noninvasive way in humans.

PURPOSE OF THE PRESENT STUDY

The compensatory vasodilation hypothesis may well be important to the issue of the mechanism of COHb effects (or non-effects) on brain function. No recent data are available on brain vasodilation due to COHb elevation in humans. Thus, it is not known if humans exhibit the same extent of compensatory response as do dogs and sheep.

The present study was designed to (a) devise a modern, non-invasive method of estimating human BBF (b) expose humans to CO while measuring BBF and (c) compare results to data in dog. Even though the eventual application of BBF data would be to the prediction of behavioral effects, no behavioral data were collected in this study. It was not deemed prudent to obtain behavioral data until the newly-designed cranial impedance plethysmograqph) (CIP) instrument and methods had been demonstrated to yield reliable and plausible results.

METHODS

SUBJECTS

Subjects were 15 men, aged 18.8 - 33.6 yrs (mean = 25.1, SD = 4.52). Subjects weighed from 68.0 - 92.5 kg (mean = 80.6, SD = 7.50), and were from 170.2 - 189.2 cm tall (mean = 179.3,

SD = 5.70). Recruitment for participation was by public advertisement. Each subject was paid $36 plus travel costs for participation. Informed consent was obtained by written statement and by oral exchanges. For the protection of human subjects, the investigators have adhered to the policies of applicable Federal Law 45CFR46. The protocol was approved by Human Use Review Office of the Department of the Army, Office of the Surgeon General, by the Committee on the Protection of the Rights of Human Subjects of the School of Medicine, University of North Carolina at Chapel Hill as well as by the Research Ethics Committee of the Department of Psychology of the University of North Carolina at Chapel Hill.

INSTRUMENTATION

Cranial Impedance Plethysmograph (CIP). The instrument which was used to estimate human BBF used the impedance plethysmograph principle. Earlier versions of such instruments, also called rheoencephalographs (Jenkner, 1962), were unstable and difficult to use. The present CIP instrument was developed jointly by the second author of the present report and personnel at the Research Triangle Institute in Research Triangle Park, NC. Modern solid state technology was used and, as a result, the device is extremely stable, reliable and simple to use. A detailed description and evaluation of the CIP is given in the Appendix. The principles of operation of the CIP are discussed in the following paragraphs.

The impedance of the cranium fluctuates in a pulsatile manner, synchronized with the cardiac cycle. Figure 1 is a plot

of the left and right CIP wave along with the electrocardiogram (ECG). Quantitative measures derived from the CIP have been developed by various investigators and related to BBF by comparison with other, more standardized, measures. Quantification is usually preceded by ensemble averaging of the CIP waves to reduce the influence of artifacts and presumably random deviations which occur from wave to wave. Measures are then performed on the ensemble-averaged CIP wave. The following is a brief review of the relevant literature.

Jacquy et al. (1974) defined a derived CIP measure and compared it to measures of BBF made simultaneously on the same human subjects via the xenon clearance method. The derived measure (F), a descriptor of brain blood flow, was made in 37 persons whose BBF was manipulated with injections of papaverine and by CO_2 inhalation. F was calculated from measures made on the ensemble-averaged CIP waveform and its first derivative as shown in Figure 2. The CIP was averaged over 30-100 cycles. The equation for F is given as follows, without the calibration coefficients, which were eliminated for clarity.

$$F = [A/t(A')]/T$$

Terms in the above equation are defined in Figure 2. The data revealed that F was correlated with the xenon clearance measure of gray-matter BBF ($r = 0.95$).

A simpler derived CIP measure was compared to BBF as measured by a radioisotope venous dilution method using 20 patients of various cerebral ischemic disturbances (Hadjiev, 1968). The CIP quantification consisted of the time to peak

divided by the total wave time as measured on the un-averaged CIP wave. The ratio was averaged for five cycles of CIP waves for each subject. The two measures of BBF were not conducted simultaneously but in close temporal contiguity. The measures correlated well ($r = 0.81$). Possibly the correlation was not as high as that demonstrated by Jacquy et al. (1974) because of (a) the fewer cycles averaged (b) the non-simultaneous measurement of BBF by the two methods (c) the possibly lower range of BBF values which occurred in the patients or (d) the different derived measure of CIP which was used.

BBF as measured by the xenon clearance method was compared to CIP quantified from the ensemble average of 16 waves (Colditz et al., 1988). Subjects were nine infants on artificial ventilation whose $PaCO_2$ altered their BBF. The amplitude of the averaged CIP was used as the measure. A low correlation ($r = 0.67$) was observed. Such a low correlation could have resulted from (a) the small number of cycles averaged (b) the small number of subjects studied (c) the possibly small range of BBF which occurred or (d) the particular derived measure of CIP may have been innapropriate.

No absolute value of BBF can be computed from the impedance measure, but presumably relative changes are accurately portrayed. Thus, if a measurement of CIP is taken as a baseline, before administration of a substance which may be a cerebral vasodilator (e.g. CO), and the measurement is repeated while the subject is under influence of the vasodilator, then the relative change in the CIP measure is the same as the relative

vasodilation. The foregoing is true if the measure of CIP is a linear function of BBF. The assumption of linear relationship to BBF appears to be valid for the measures of CIP proposed by Jacquy et al. (1974).

From the positioning of the electrodes and knowledge of cranial anatomy, most of the variation in the CIP may be assumed to be due to BBF although contamination by extra-brain (scalp) blood flow is possible. The effect of extra-brain blood flow contamination of the impedance measurement depends upon the relative volume of such flow and its variability with respect to COHb. The following is a logical evaluation of the possible effects of extra-brain blood flow on the CIP. If extra-brain blood flow does not vary as a function of COHb, or varies in the same way as BBF, then it will not affect the CIP measure. If it varies inversely with BBF, then the measurement of impedance would be attenuated, but proportional to BBF. If it varies non-systematically with respect to COHb then the impedance measure would be made more variable, but the mean CIP values would still be well related to BBF. Thus, it may be argued on strictly logical grounds that even if extra-brain blood flow contamination were present in the impedance measure, the measure would still be a good analogue of BBF in terms of the mean measurement.

The influence of scalp blood flow was tested in 15 subjects by Jevning et al. (1989). They computed various measures from the averaged CIP, among which was the F measure of Jacquy et al. (1974). The CIP measures were taken with and without a constrictive band around the head to temporarily stop scalp blood

flow. Differences between conditions were extremely small, indicating that scalp blood flow was a negligible component of F.

Display and Recording. The pulsatile signals of the CIP were displayed along with the ECG and an eye movement channel, on a Grass model 7 D polygraph with the low frequency time constant set at 0.15 Hz (half amplitude point) and high frequency filter set at 40 KHz. The high frequency cutoff is not critical beause the output of the present CIP instrument contains so little noise. The outputs of the two Grass amplifiers for the CIP were digitized by a 12-bit analog-to-digital converter at the rate of 100 samples per sec. Digitized data were stored on disk in an IBM PC/XT. Computation of F was performed by the IBM PC/XT via an offline program written by the senior author of the present document.

CO EXPOSURE

During the experiment each subject breathed sufficient CO designed to produce one of three approximate COHb levels; endogenous, 12.5 or 17.0%. Exposure to CO was achieved by having subjects breathe air/CO mixtures from a Douglas bag. The concentration in the bag was fixed for each of three groups at either 0, 6,000 or 9,600 ppm. The total bag volume was 30 l. No effort was made to achieve particular COHb levels for each person by, e.g., using physiological variables to compute the amount of CO required. It was considered desirable, for regression analysis purposes, to have a more-or-less continuous distribution of COHb than to achieve 3 distinct groups. Bags were prepared by an experimenter who had no contact with the

subject. Neither the subject nor those experimenters in contact with the subject were informed of the bag contents until after the experiment.

PROCEDURE

Before being accepted into the study, potential subjects were given routine physical examinations with special attention to cardiac health. Following this, if no problems were detected, they were given a twelve-lead resting ECG. If no problems were noted, they were given a standard Bruce exercise ECG. Only persons who showed no signs of abnormality of any kind were accepted for study.

Subjects arrived in the laboratory between 0830 and 0930 and were given a brief physical examination by a physician. Following informed consent, pre-exposure blood was drawn and Beckman silver-silver chloride (1 cm diameter) electrodes were attached to the subject's head using EC-2 electrode paste. Excitation signal (100 kHz, 4 ma) was applied between two electrodes, one attached immediately above inion, the other attached to the center forehead, 5 cm above nasion. Reference leads for the two impedance measurement channels were attached to each mastoid process. The two inputs of the impedance measurement channels were attached to the forehead, 5 cm above nasion and 4 cm on each side of midline.

After electrode preparation, a subject was seated in a double walled audiometric testing chamber approximately 3 x 3 x 3 m in size. To avoid movement-induced artifacts in the CIP, he was trained to relax during the measurement periods. The

relaxation training consisted of instructing him to perform a continuous mental inventory of all muscles including the tongue, ears, scalp, temples, jaw and eyes to assure that each of the muscles was relaxed and not moving. The subject was to allow his head to drop forward until his chin came to rest on his chest.

When the subject became relaxed, a pre-exposure baseline period of CIP was recorded for 2.5 min. Following the baseline recording the subject was instructed to breathe the contents of a 60 l Douglas bag which had been filled with 30 l of either air or one of two air/CO mixtures. The bag breathing required 3.0 - 6.2 min (mean = 4.2, SD = 1.1). The subject was then removed from the chamber and blood was drawn 2 min after the end of bag breathing. The subject then returned to the chamber for a post-exposure CIP measurement. The time from the end of bag breathing until the beginning of post-exposure CIP measurement was 4.55 - 8.47 min (mean = 6.15, SD = 1.0). Note that no information can be gleaned from the present design regarding the persistence of the compensatory BBF response because only short term CO exposure was used.

The subject then exited the chamber and electrodes were removed. If his COHb was above 10%, he was requested to breathe normobaric O_2 for up to 1 hr until his COHb was reduced to less than 10%. Blood samples were drawn as appropriate to assure COHb reduction. After reduction of COHb to less than 10% the subject was released with the admonition to avoid even moderate exercise and activities which required alertness or fine motor performance.

CIP DATA REDUCTION.

The value F of Jacquy et al. (1974) was computed on the ensemble-averaged CIP data from the present experiment. The equation for F and its interpretation is given above in the paragraph entitled "INSTRUMENTATION", "Cranial Impedance Plethysmograph (CIP)". One value of F was computed for each subject and each condition in the experiment.

Ensemble averaging was performed off-line to allow adequate artifact rejection. During averaging each cycle of the two-channel CIP was displayed on a CRT screen and the operator was then required to determine whether the displayed waves were contaminated by artifact or distorted in a number of ways (see below). If no problems were found, the data were passed to the routine for computation of the ensemble averages. Before inclusion into the ensemble average, a straight line was fitted between the beginning and end points of the CIP wave. The straight line was then subtracted from the wave to eliminate baseline drift. When the number of required cycles was reached, the averaged cycles were quantified by computation of F.

The criteria for acceptance of the displayed waves were (a) the dicrotic notch (see Figure 1) had to be present (b) no truncation or clipping could be present (c) the falling portion after the dichrotic notch had to be relatively linear and (d) there could be only moderate baseline drift (maximum of approximately 30 degree baseline angle with respect to horizontal). The baseline drift was assessed by connecting an imaginary line between the beginning and end points of the wave.

The criteria are qualitative and therefore difficult to specify exactly. The reliability of the selection procedure was tested by having all records analyzed by two independent observers who were blind to the exposure conditions. The correlation between their results was r = 0.95. Thus, the procedures were demonstrably reliable.

For each subject and both conditions, the ensemble average contained 50 cycles of data. Derived measures (F) were computed for the left and right channels and were considered to be measures of BBF for the left and right sides of the head. The final measures of vasodilation were computed as the relative increase of F from pre- to post-exposure for left and right channels. The relative increase in F will be called the relative increase in brain blood flow, R(BBF). This procedure avoids absolute units of flow and is the same as that used in the literature in dogs and sheep (Jones & Traystman, 1984) except that the units in the literature are usually expressed as percentages.

Computational steps involving CIP quantification can be summarized as follows:

(a) Select undistorted CIP cycles.

(b) Subtract baseline drift from each acceptable pair of CIP waves (left and right channels).

(b) Compute ensemble average CIP cycle for both channels.

(c) Compute F for each 50-cycle ensemble averaged CIP wave.

(d) Average F across both channels to produce one measure per subject, per condition. Fewer measures were desired to simplify the hypothesis testing. Individual channels were later analyzed in an exploratory manner.

(e) Compute R(BBF) from pre to post exposure.

After hypothesis tests were made, changes were made in the above procedure and the data were reanalyzed on an exploratory basis. The purpose of the reanalyses was to explore the sensitivity of the results to the data reduction methods.

STATISTICS.

A straight line was fitted to R(BBF) as a function of COHb using BMDP statistical software for microcomputers (Dixon, 1988), program 1R. The significance of the fit was evaluated by the F test as computed by program 1r. The alpha level was 0.05.

During exploratory analyses, regression solutions were compared for significant differences using methods from Kleinbaum, Kupper and Muller (1988, p 266-269). Reliability of R(BBF) estimation methods was analyzed as a function of the number of CIP cycles included in the ensemble averages by use of the Spearman-Brown equation (Ghiselli, 1964).

RESULTS

A total of seven statistical analyses were conducted, each of them to test a particular hypothesis. One of the hypotheses was formed on an a-priori basis, the others were exploratory. Table 1 is a list of the hypotheses and the results of their

tests. In the following text the hypothesis numbers will be used for reference to Table 1.

R(BBF) AS A FUNCTION OF INCREASED COHb (ΔCOHb).

Figure 3 is a plot of R(BBF) for each subject as a function of that subject's ΔCOHb. The line in Figure 3 is a regression line the particulars of which are listed in Table 1, hypothesis 1A. The fitted line accounted for a significant amount of variance ($r = 0.62$, $p = 0.019$).

EXPLORATORY EXPERIMENTS AND ANALYSES.

Comparison of Human and Dog Data. To compare the human results with those from experiments on dogs, raw data were obtained from Richard Traystman. Figure 4 is a plot of dog R(BBF) as a function of ΔCOHb. Exploratory regression analysis (see Table 1, Hypothesis 1E) yielded $r = 0.47$, $p = 0.087$. The experimental design employed by Traystman was to use each dog as his own control for 1 or 2 elevated COHb measures. Consequently, there were no independent pre-exposure baseline data as there were for the human data. It is probable that the non-significant result from hypothesis 1E was due to the fact that the pre-exposure data were not available thus reducing the range of ΔCOHb. For exploratory purposes, it was decided to use the fitted function given in Table 1.

It was desired to explore the possibility that the functions fitted to the human data and the dog data did not differ significantly. Slopes and intercepts of the dog and human data were tested for significant differences (Kleinbaum et al., 1988, pp 266-269). The two intercepts were not significantly

different, $p > 0.5$ (see Table 1, hypothesis 2E). The two slopes did not differ, $p > 0.1$ (see Table 1, hypothesis 3E). Thus there was no evidence that the lines of best fit for human and dog R(BBF) were different. Qualitatively, the intercepts were nearly the same. The slopes had quite different values (0.0403 vs. 0.0135). The lack of a significant difference in the slopes, however, implied that there was a large uncertainty about one or both of the estimates.

Because the two lines cannot be said to differ, a single function was fitted to the pooled data from the human and dog experiments (Figure 5). The regression analysis yielded $r = 0.69$, $p < 0.0001$ (see Table 1, hypothesis 4E). The slope and intercepts of the fitted function most closely approximated that of the dog data.

Number of CIP Cycles Required. In the above analyses of the human CIP, the ensemble average contained 50 CIP cycles. From an efficiency standpoint it would be desirable to use as few cycles as possible. Having to average fewer cycles would also mean that a shorter length of raw CIP data would be needed and therefore more temporal resolution in an ongoing record of BBF could be obtained. Too few cycles, however, might yield unreliable results.

To determine the loss of stability (reliability) as a function of the number of cycles averaged, the entire data set was analyzed twice, using only 10 CIP cycles in the ensemble average (10 CIP cycles in each channel and the results from the two channels averaged). The first 10 cycles of CIP came from the

early part of the 2.5 min block of data and the second 10 cycles came from a later part. Pre- and postexposure records were analyzed to produce early and late estimates of F and from these the R(BBF) was computed for both early and late parts of the record. Correlation between the early and late estimates of area (the reliability coefficient) was 0.71, $p < 0.007$ (see Table 1, hypothesis 5E).

The Spearman-Brown formula (Ghiselli, 1964) was used to estimate the reliability for various numbers of CIP cycles in the averaging. Figure 6 is a plot of the estimated reliability of the R(BBF) as a function of number of CIP cycles, from 10 to 150 cycles. As can be seen, the reliability of the 50-cycle data as used in the analysis of the above experiment, was estimated to be 0.92. Fewer CIP cycles in the average would be expected to yield lower reliabilities and therefore greater variance in estimates of area across conditions or individuals.

The intercept for the fitted line to relate the two 10-cycle estimates of R(BBF) was near zero (0.078). The slope of the line was 1.0294, (nearly unity). It appears that the two estimates not only correlated well but were nearly identical.

Differences between channels. To compare information between left and right channels, the R(BBF) was not averaged across the two channels. When data from the 50-CIP-cycle analysis was used, the correlation between left and right channel R(BBF) was $r = 0.90$ (see Table 1, hypothesis 6E). Apparently, there is no variation due to ΔCOHb in one channel that is not found in the other.

DISCUSSION

R(BBF) AS A FUNCTION OF ΔCOHb

The R(BBF) as computed in the present study is significantly related to ΔCOHb. The correlation is approximately 0.62. Observations about R(BBF), based upon the present results, are potentially important. From Figure 3, it may be seen that in the unexposed control group, the BBF strongly tended to decline from the baseline to the "post-exposure" measurement even though there was no change in COHb. This change might reflect a decreased brain activation due to reduced anxiety about the exposure or the experimental procedures in general or a reduced level of stress. No data are available from which to deduce the cause of the decreased BBF. The amount of BBF increase for the CO-exposed subjects must, therefore, be compared to the predicted value for the unexposed controls at an equivalent time in the experiment since without COHb, they too would presumably have exhibited vasoconstriction. The best measure of effect is the difference between the control and the experimental groups, not the absolute vasodilation of the experimental group, since the latter is the due to the contribution of the independent variable plus whatever other variables are also acting on the control group.

From Figure 5, it is apparent that many of the subjects (both humans and dogs) do not appreciably increase BBF over control values after elevated COHb. The regression line slope is significantly different from zero because most of the subjects respond. Apparently, some subjects do not exhibit appreciable brain vasodilation in the presence of COHb. The variation in BBF

response may be due to individual differences, or to some other unknown factor. Since there is high variation in BBF responses Assumption 3 in the Introduction may not hold, i.e. there may be variation which is related to the individual or to the occasion of measurement. The apparent nonresponders and (for that matter) the apparent over responders may, of course, be entirely due to random variation in the measurement. No conclusion can be drawn without further data.

IMPLICATION OF OBSERVATIONS ABOUT BBF ON BEHAVIOR

Unpublished observations (see Introduction) imply that only some subjects' behavior may be affected by COHb. The present results imply that the same may hold for the BBF compensatory response. It seems to be a reasonable hypothesis that the behavioral effect of COHb is inversely related to the BBF compensatory response. The subjects who exhibit adequate BBF compensation for COHb may show no behavioral effects while those subjects that do not adequately vasodilate may be behaviorally impaired. If the above hypothesis is valid, much of the variance in the behavioral effects of CO exposure may be accounted for by BBF variation. It could also be true that there exists a population at increased risk of behavioral impairment by COHb due to a failure to compensate by vasodilation. Such information could be quite important if, indeed, the observed variation in R(BBF) is other than random error of measurement.

HUMAN VS. DOG R(BBF)

It appears that the measure of R(BBF) used in humans in the present study yields data which are similar to the R(BBF) data

reported by Jones and Traystman (1984) in dogs. The slope of the function derived from humans does not differ significantly from that of dogs even though the numeric value of the slopes differed markedly (human slope greater than dogs'). The numeric difference between slopes could easily be due to the small range of COHb values in the two studies, which could have resulted in erroneous estimates. The slope of the human data could have also been over estimated because of a single subject whose R(BBF) was 2.28 for a ΔCOHb of only 9.8% (a possible outlier). Conversely, the slope of the dog data could have been erroneously estimated because of the absence of independent baseline data thus leading to the absence of control data at near-zero ΔCOHb. When the two data sets were combined, however, the function fitted for the pooled data was much more similar to the dog than the human function. While no unique conclusion can be reached, it appears that the slope of the human function was overestimated due to a possible outlier. If the variation across subjects is not due to random error (see above) the slope of the human function may not be important anyway, because in the face of, e.g. real individual differences, a regression analysis would be less appropriate.

Qualitatively, the scatter of the dog data is similar to the scatter of the human data. The similarity of the two data bases, the well-understood principles of impedance plethysmography and the standardization of CIP against other measures of BBF (Jacquy et al., 1974), lend credence to the use of CIP as a measure of relative BBF.

OTHER EXPLORATORY RESULTS

There seems to be no difference between the measured response in left and right channels of the CIP to ΔCOHb. This is due either to the lack of lateral asymmetry in the brain circulatory system or the lack of the ability of the device to resolve lateral differences. From the present data base, there is no way to decide between the above alternatives.

The number of cycles of CIP over which averaging is carried out is important to the reliability and stability of the estimates of R(BBF). Figure 6 indicates that approximately 40 cycles must be averaged to obtain a reliability coefficient of 0.90. This figure is very useful for experimental design purposes. By use of Figure 6, estimates of the duration of measurements (via number of CIP cycles to average) in a particular condition can be made, reliabilities can be estimated and one aspect of the power of a test can be determined.

CONCLUSIONS

(1) The reliability of the CIP, using the methods of Jacquy et al. (1974) to measure BBF as an analog of cranial impedance, is high when sufficient cardiac cycles of data are included in the ensemble average.

(2) The BBF as measured by the CIP in man compares well with other measures of BBF in man and with BBF measures made by other methods in dogs.

(3) The CIP did not show lateralization in the BBF response to COHb.

(4) Average BBF increases with COHb in dogs, sheep and humans. There is, however, a large amount of variability across individuals and/or occasion of measurement.

TABLE 1. SUMMARY OF THE HYPOTHESES TESTED WITH TEST STATISTICS

H#[1]	TEST	STAT.	TEST df	p	r	int	slope
1A	R(BBF) = f(ΔCOHb) (HUMANS)	F=7.34	1,12	0.019	0.62	0.893	0.0403
1E	R(BBF) = F(ΔCOHb) (DOGS)	F=3.48	1,12	0.087	0.47	0.987	0.0135
2E	HUMAN AND DOG INTERCEPTS ARE SAME	t=0.28	24	>0.50	n/a	n/a	n/a
3E	HUMAN AND DOG SLOPES ARE SAME	t=1.59	24	>0.10	n/a	n/a	n/a
4E	R(BBF) = F(ΔCOHb) MAN, DOG DATA POOLED	F=23.2	1,26	<0.001	0.69	0.985	0.0139
5E	REPEAT ANALYSIS FOR RELIABILITY (TEST 1)	F=10.9	1,12	0.007	0.71	0.078	1.0294
6E	DIFFERENCES BETWEEN LEFT/RIGHT R(BBF)	F=49.9	1,12	<0.001	0.90	0.246	0.7903

[1]Hypothesis numbered 'A' was a priori. Those numbered 'E' were exploratory.

GLOSSARY

Blind	An experimental strategy in which a participant is kept uninformed about a condition. Also see "Double Blind".
Digitization	Conversion of a continuous signal of function to a discrete number series.
Double blind	An experimental strategy in which neither the subject nor the experimenter in contact with the subject is informed of the conditions of exposure. The strategy is used to minimize bias due to expectations.
Plethysmograph	An instrument used to infer changes in blood flow in an intact organ of the body from changes in e.g. volume, electrical impedance, optical or acoustic properties of the organ.

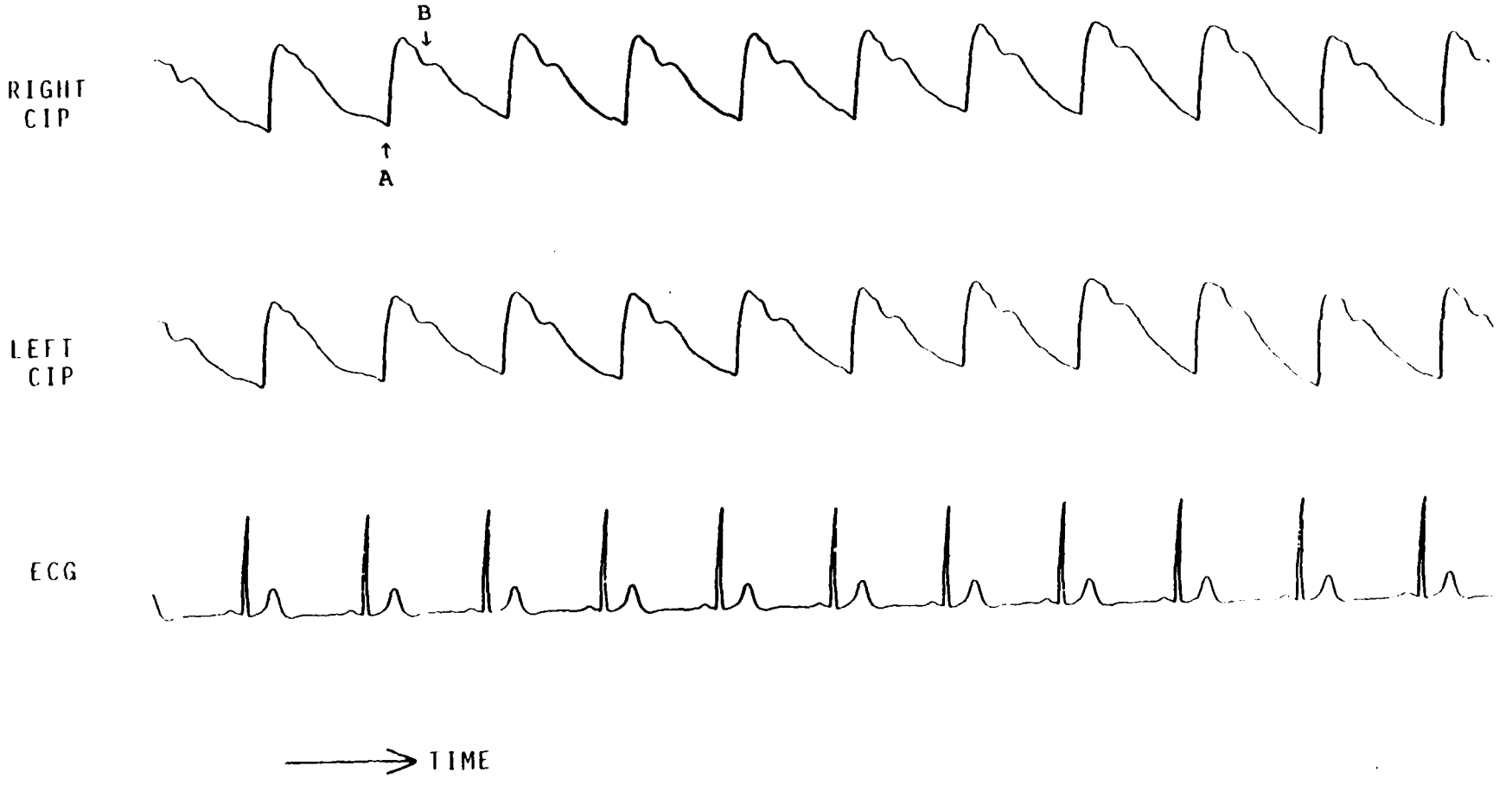

Figure 1. Plots of the two-channel CIP and the ECG. The left and right CIP refer to the left and right sides of the head. On each CIP cycle, points typical of point A correspond to the opening of the aortic valve while poits typical of point B are the dichrotic notch, corresponding to the closing of the aortic valve.

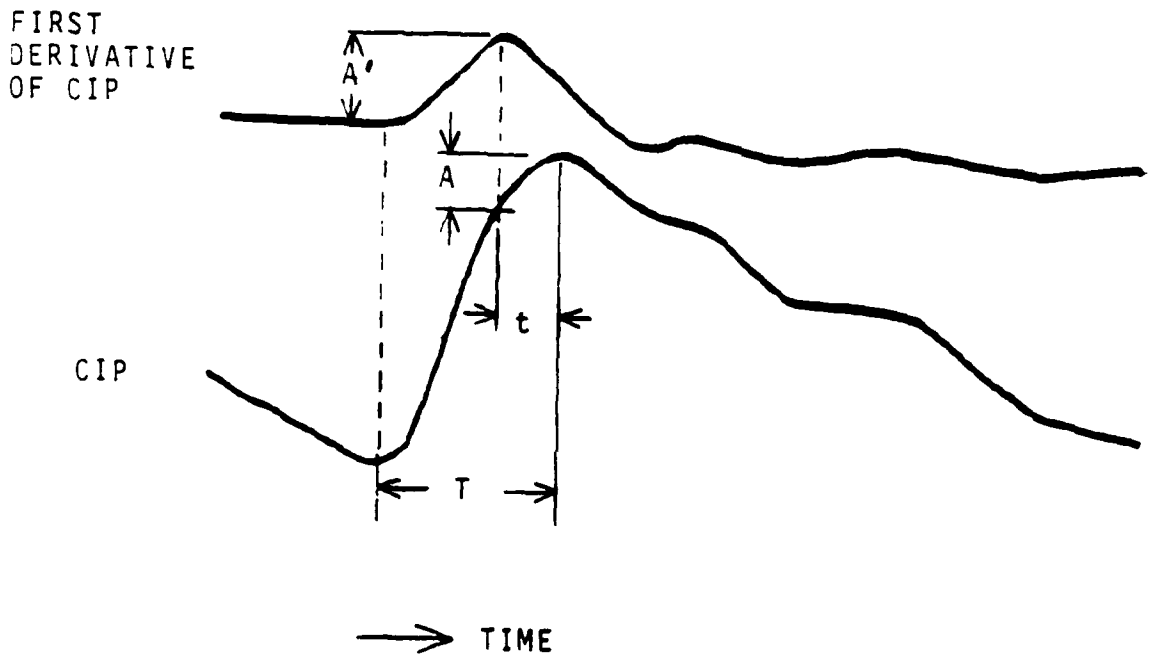

Figure 2. Diagram of the CIP wave and its first derivative, showing the measurements of A, A', T and t. These measurements are used to compute the derived measure, F, as defined by Jacquy et al. (1974). The equation for F (without calibration coefficients) is $F = [A/t(A')]/T$.

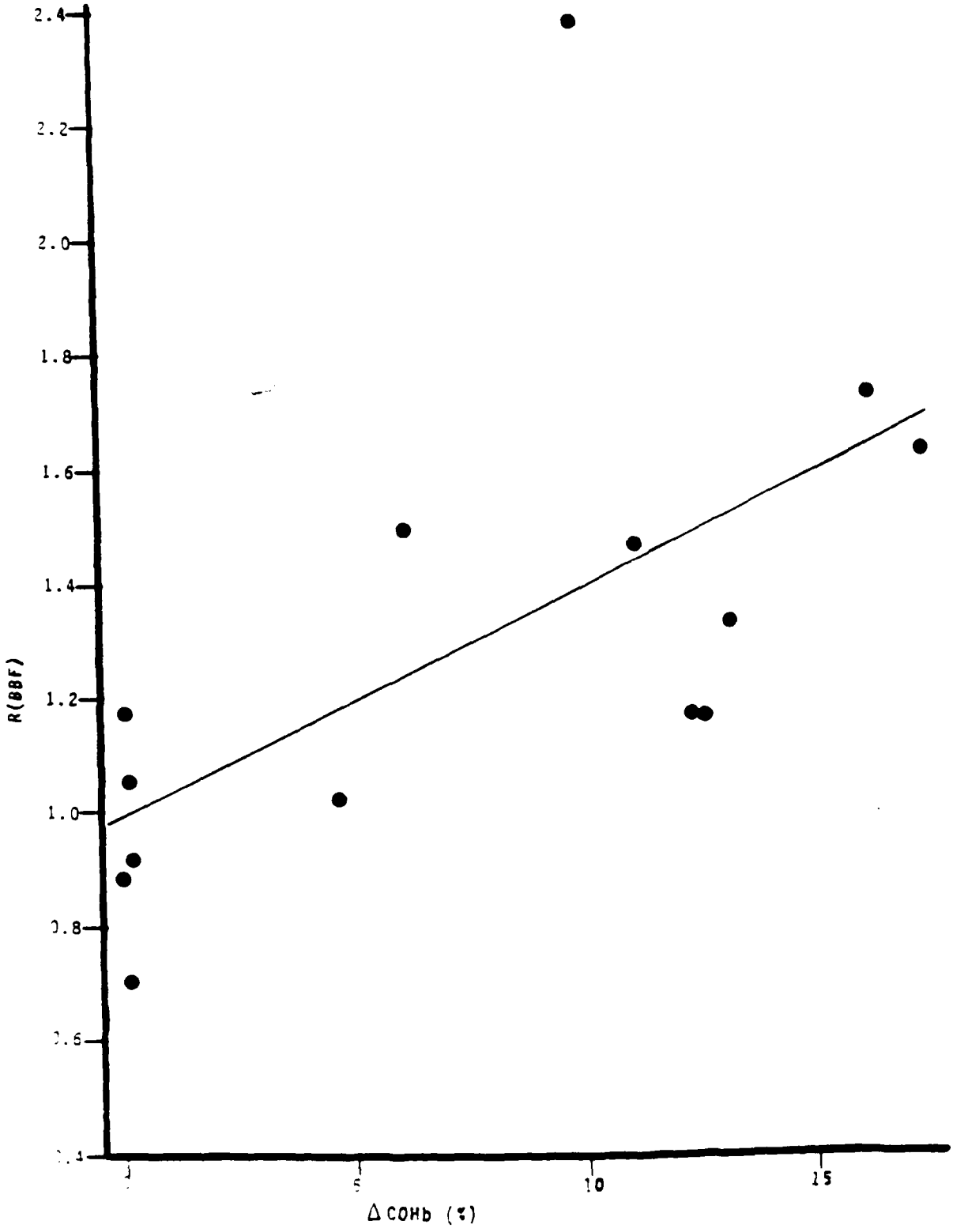

Figure 3. Scatter plot and fitted function for the values of R(BBF) as a function of ΔCOHb for 14 men. The regression line was R(BBF) = 0.0403(ΔCOHb) + 0.89. r = 0.62, p < 0.019.

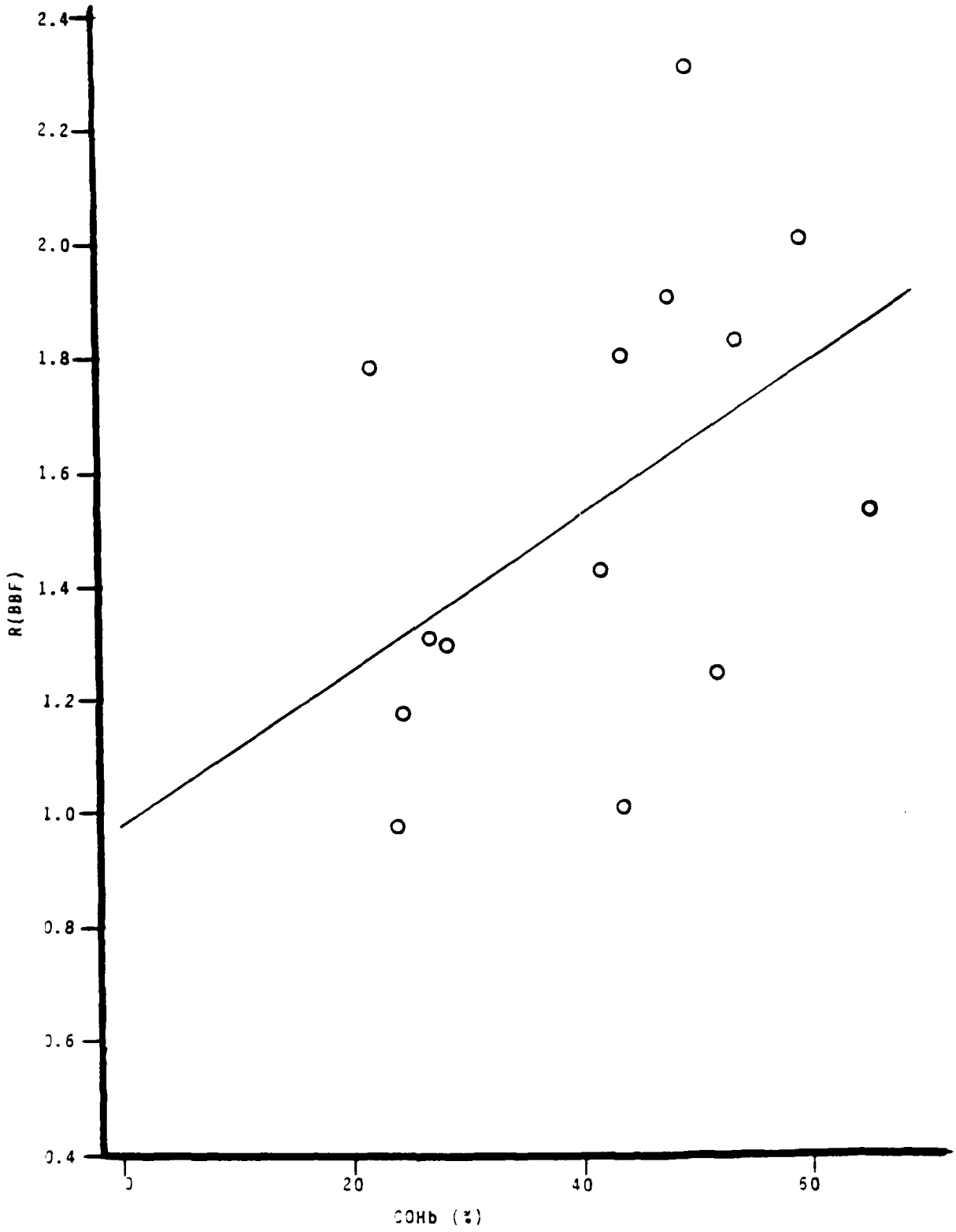

Figure 4. Scatter plot and fitted function for the values of R(BBF) as a function of ΔCOHb for 14 dogs. The regression line was R(BBF) = 0.0135(ΔCOHb) + 0.99. $r = 0.47$, $p < 0.087$.

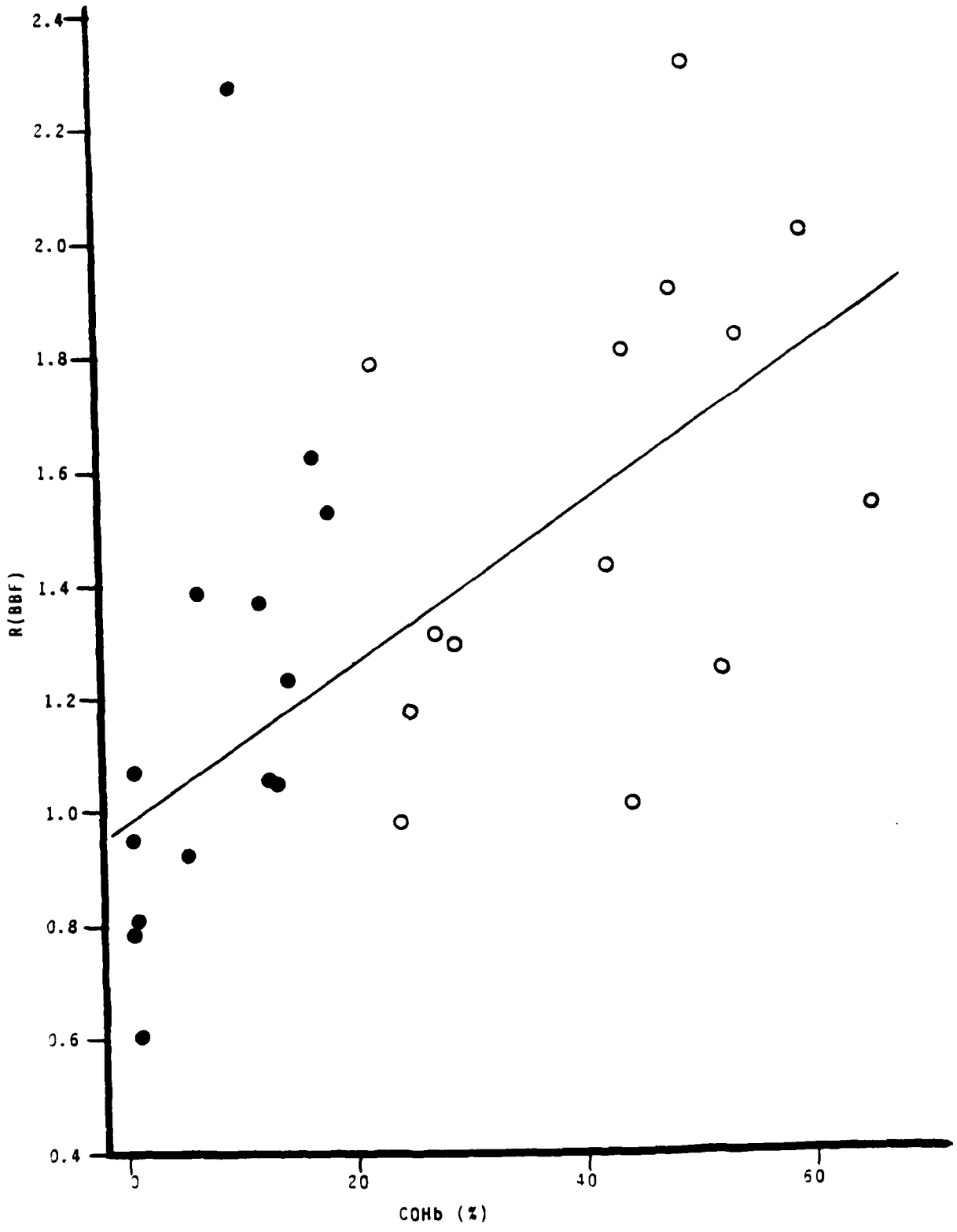

Figure 5. Scatter plot and fitted function for the values of R(BBF) as a function of ΔCOHb for the pooled data of 14 men and 14 dogs. Open circles are dog data, filled circles are human data. The regression line was $R(BBF) = 0.0139(\Delta COHb) + 0.99$. $r = 0.69$, $p < 0.0001$.

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APPENDIX

TECHNICAL DESCRIPTION OF THE CRANIAL IMPEDANCE PLETHYSMOGRAPH (CIP) INSTRUMENT

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TECHNICAL DESCRIPTION OF THE CRANIAL IMPEDANCE PLETHYSMOGRAPH (CIP) INSTRUMENT

INTRODUCTION

Noninvasive measurement of cerebral blood flow can be accomplished by a variety of medical instrumentation, i.e. nuclear magnetic resonance (NMR), radio labeled isotopes in the blood, and doppler backscatter of red blood cells exposed to ultrasonic signals. Each method has its advantages, disadvantages, cost effectiveness and the type of information obtained. Each method is unique and may be favored over other methods depending on the desired application. i.e., clinical diagnostic, research, mobility, and various environmental exposure situations.

Of the popular methods, (NMR) requires large size buildings and computer controlled data acquisition systems. Other systems include mobile carts which are far too costly and not suitable for laboratory experiments or field applications. Alternative noninvasive measurements of cerebral blood circulation have been considered which are more cost effective and physically portable. For the latter, the level of measurement accuracy and validation is not equivalent to the more sophisticated and costly methods.

For the purposes of behavioral experiments involving carbon monoxide exposures with (COHb levels of 10-20%), the impedance plethysmographic method was selected for measurement of cerebral pulsatile blood volume. This method is also known as rheoencephalography (Jenkner, 1962). Commercially available instruments, however, were found to be inadequate for one or more

of the following reasons: (a) poor sensitivity to cerebral vessel vasomotor events, (b) poor signal to noise ratio, (c) D.C. drift problems, (d) excessive artifact, (e) excessive size, (f) poor electrical safety characteristics, and (g) poor calibration and data display methods.

In order to overcome the above limitations, a dedicated two channel cranial impedance plethysmograph (CIP) instrument was designed, and fabricated. The CIP, as a result of its basic design, measures the change of electrical impedance (z) of pulsatile red cell masses as they pass between specific excitation (current source electrodes) and detection electrodes (Jenkner, 1962). Briefly, the measurement of z-changes between electrodes represent changes of electrical conductivity in a 100 KHz electrical field. This measurement, therefore, is not a direct measure of any fluid flow component such as blood. Conversely, the measured signal amplitude is generally proportional to the fluid bolus profile.

SYSTEM DESCRIPTION

The CIP system used in the present study is comprised of (1) a two channel impedance plethysmograph instrument, (2) a two channel electrode system, (3) an auxiliary ECG channel and (4) a power source. The CIP electrodes are placed in proximity to the area of observation (cerebral vascular tree). One pick-up electrode (+) channel (1) is placed over the right eyebrow. A second pick-up electrode (+) channel (2) is placed over the left eyebrow. Each CIP channel is referenced (-) to each mastoid process. A 100 KHz, 4 ma square-wave excitation signal from a

constant-current source is applied, to the center of the forehead. The excitation current source is referenced to a common electrode on the inion (back of the skull).

This electrode configuration creates a 100 KHz square-wave field within the frontal to occipital skull region. As pulsatile blood flow occurs, the 100 KHz signal is modulated and picked-up by the (±) recording electrodes. The modulated signal is subsequently processed to its analog slow-wave component. As cerebral vascular modulation takes place, this change of impedance is amplified and sent to a synchronous detector circuit. The synchronous detector removes the 100 KHz (carrier) signal but retains its slow-wave envelope as an analog signal. The analog signal reflects the pulsatile blood volume profile including the opening of the aortic valve and the dicrotic notch or closing of the aortic valve (see Figure 1).

The analog signal is fed to a delta z rebalancing circuit which has a maximum D.C. limit in the event of artifact, i.e., if a lead movement, scalp flexing signal exceeded the delta z threshold, the circuit is automatically re-zeroed. This prevents overdriving or saturation of any recording device or analog to digital converters connected to the CIP output. Each of two CIP channels are virtually identical.

IMPEDANCE DISPLAYS AND OUTPUT CONNECTORS

Each channel contains its own LCD display for calibration and subject impedance measurements. Each channel has the following analog signal outputs:

1. Zo = base impedance
2. Z = subject impedance
3. dz/dt = 1st derivative impedance
4. ECG = electrocardiogram

The detected (Zo) signal is also sent to a D.C. 16 Hz low pass filter and to an isolation amplifier. Additionally, the output of the rebalancing (z) signal is fed to an isolation amplifier. When velocity of flow profile information is desired, the (z) is fed to a differentiator amplifier and then to an isolation amplifier, resulting in a dz/dt signal.

In addition to the impedance signals, a three lead ECG signal is provided to check for cardiac rhythm synchronization with CIP signals. The output connectors provide signal levels of +1.5 volt for use with an analog recorder, or an analog to digital converter.

SUBJECT ELECTRICAL SAFETY

All bioimpedance signals and the ECG signal are fed to output connectors via isolation amplifiers. The isolation amplifier provides low leakage currents and reduce possible shock hazards when the CIP is connected to the test subject or other instruments.

FRONT PANEL CONTROLS

A. Power Switch - This switch turns the instrument off and on. When power is on the red POWER light is lit.

B. Z0 Meter - This meter indicates what has traditionally been called the "base" impedance or the "static" impedance of the CIP signal. The meter reads 0 to 70 ohms +1% of reading +0.1 ohm.

C. Operate-Calibrate Switch - This is a momentary-contact, center-off switch which selects the operating mode of the instrument. Momentarily pushing the switch in the Operate direction connects the instrument to the front panel input connectors during which the calibrate light is not lit. Momentarily pushing the switch in the calibrate direction connects the instrument to the internal calibrator signals during which the calibrate light is lit.

D. Rebalance Switch - This switch has three positions AUTO, OFF, and ZERO.

1. AUTO - In this position the instrument automatically rebalances (re-zeros) the delta Z channel whenever the threshold which has been set on the front panel dial is exceeded. When rebalancing occurs, the rebalance light on the front panel flashes and a 10 ms TTL pulse appears at the rebalance output connector on the rear panel.

2. OFF - This position sets the rebalance threshold to +12V (+3 ohm). Under normal signal conditions this effectively inhibits all rebalancing. (NOTE: Re-balancing may still occur if the input is open circuited and the delta Z channel saturates with noise.)

3. ZERO (Momentary) - This position forces a rebalance to occur by momentarily setting the rebalance threshold to 0 volts.

E. Rebalance Threshold Control - This ten-turn, continuaous dial indicates the relative rebalance threshold on a scale of 0 to 10. This corresponds to an impedance range of 50 milliohms (0) to 0.5 ohm (10.)

F. Z Input Connector - 9 pin input connector for the subject impedance leads.

G. ECG Input Connector - 5 pin input connector for the subject ECG leads.

REAR PANEL CONNECTIONS:

ECG Output - BNC

dZ/dt Output (CH1 & CH2) - BNC

delta Z Output (CH1 & CH2) - BNC

Z0 Output (CH1 & CH2) - BNC

Rebalance Pulse Output (CH1 & CH2) - BNC

INITIAL SETUP

A. Connect the instrument to a standard 3-wire 120 VAC power source

B. Turn the instrument on via the front panel power switch. The red POWER light should light.

C. Switch both channels (1&2) to the calibrate mode by momentarily pushing each operate-calibrate switch to the calibrate position. Both red calibrate lights should light.

D. Rebalance both channels (1&2) by momentarily depressing each rebalance switch to zero position. If a rebalance operation occurs the green rebalance light should flash.

E. Display the calibration signals. They should be as follows:

ECG	- 2.0Vp-p Triangle Wave
dZ/dt	- 4.0V0-0 Square Wave
delta Z	- 680mVp-p Triangle Wave
Z0	- 0.0 volts
Rebalance Pulse	- 0.0 volts except during rebalance when each rebalance will generate a 5V, 10 ms pulse.

F. Front Panel Meters - With the instrument in calibrate mode both meters should indicate 35 ohm (+0.45 ohm).

OPERATION

A. Attach the CIP electrodes to the subject and connect them to the Channel 1 input cable. The connections to this cable are numbered as follows:

1 Current source

2 + Impedance Pickup

3 - Impedance Pickup

4 Current Return (common)

Connect the Channel 1 cable to the front panel of the instrument. NOTE: If only one impedance channel is being used it must be channel 1 because only channel 1 is connected to the current source.

B. If two impedance channels are being used, attach the second set of CIP electrodes to the subject and connect them to the Channel 2 input cable. The connections to this cable are numbered:

1 OPEN

2 + Impedance Pickup

3 - Impedance Pickup

4 - OPEN

Connect the Channel 2 cable to the front panel of the instrument. NOTE: Channel 2 does not contain a current source. It must be used in conjunction with the Channel 1 current source to make a measurement. Similarly, if Channel 1 is switched into the calibrate mode the current source is removed from the subject and Channel 2 will not record a signal even though it remains in the operate mode.

C. Attach the ECG electrodes to the subject and connect them to the input cable. Connect the ECG cable to the front panel of the instrument.

D. Switch the impedance channels being used from the Calibrate to the Operate mode. Again, if a single channel is being used it must be Channel 1. Momentarily depress each rebalance switch to the Zero position then set them to the AUTO position. Set the rebalance threshold dial to 10. It is best to leave the threshold set on 10 as this allows the largest delta Z signal possible without saturating the dZ/dt circuits.

E. Connect an oscilloscope or strip chart recorder to the outputs and observe subject waveforms.

SPECIFICATIONS

I. CURRENT SOURCE

Frequency	100 kHz, sinusoidal
Output level	4 mA rms
Effective output impedance	50 kohm
Dynamic range	0 ohm to 2 kohm
Maximum open circuit output voltage	+12V

II. Zo Channel

Output sensitivity	200 mV/ohm
	0 ohm = -7.0 V
Bandwidth (-3dB)	DC to 0.16 Hz
Dynamic range	0 ohm to 70 ohm
Noise	2mVp-p
Output Impedance	50 ohm

III. delta Z Channel

Output sensitivity	8 V/ohm
Bandwidth (-3dB)	DC to 100 Hz
Dynamic range	+3 ohms (Rebalance off)
Noise	4mVp-p
Output Impedance	50 ohm

IV. dZ/dt Channel

Output sensitivity	2 V/(ohm/sec)
Bandwidth (-3dB)	2 Hz to 40 Hz
Dynamic range	+Vp-p 0-40Hz (2.5 mohm/sec equivalent)
Output Impedance	50 ohm

V. ECG Channel

Output sensitivity	1000 V/V
Bandwidth (-3dB)	0.07 Hz to 106 Hz
Dynamic range	+12 mV input
Noise	4mVp-p output
Output Impedance	50 ohm

VI. Front Panel Meter

Range	0 to 70 ohms
Accuracy	+1% of reading +0.1 ohm

VII. Front Panel Threshold Potentiometer

Range	Indicator	Threshold Voltage	Effective ohms
	0	0.2 V	+50 milliohms
	10	2.2 V	+550 milliohms

VIII. Rear Panel Rebalance Pulse

TTL compatible	(0 to 5 V)
Pulse width	10 msec

IX. Internal Circuits

Synchronous Demodulator - recovers only resistive (in-phase) component

Digital Rebalance

Droop rate	0 V/sec
Resolution	16 bits (1 LSB = 763 uohm)
Dynamic range	0 to 10 V (0-50 ohm)

(Zo suppression resolution to within +6 mV at delta Z output [+0.75 milliohms])

X. Isolation

Maximum leakage current, total instrument	14uA
Maximum lead leakage current to ground	6uA
Maximum isolated lead leakage current to ground (120 VAC applied between lead and AC power ground)	23uA

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